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Postirradiation Cell Division in 5-Fluorouracil-pretreated *Escherichia coli*

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ABSTRACT

The correlation between 5-fluorouracil-induced resistance to ultraviolet (UV) light and the ability of bacterial cells to repair irradiation damage was investigated in various strains of *Escherichia coli*. Preincubation with 5-fluorouracil did not influence the dark-repair mechanism. It affected, however, the UV light-induced damage of cell division in filament-forming strains. It is suggested that the delay in postirradiation macromolecular synthesis of 5-fluorouracil-pretreated bacteria plays a decisive role in the recovery process leading to cross cell wall-forming ability in the damaged strains.

In a previous investigation (9), it was shown that *Escherichia coli* preincubated with low concentrations of 5-fluorouracil become resistant to ultraviolet (UV) irradiation. It was further shown that, in *E. coli* B, acquisition of 5-fluorouracil-induced resistance to UV irradiation appears together with incorporation of the analogue into messenger ribonucleic acid (mRNA) and accumulation of this ribonucleic acid fraction (8).

Two possibilities to explain 5-fluorouracil-induced resistance by mRNA accumulation seemed feasible. (i) Excess mRNA may protect the bacterial deoxyribonucleic acid (DNA) from the photochemical effects of UV light. (ii) It may allow repair processes to operate more effectively.

The first alternative was tested (8), and it was found that no reduction in the photochemical lesions, as assayed by thymine dimerization, occurs in 5-fluorouracil-pretreated cells, thus making this possibility unlikely. The possibility that repair processes may be affected was therefore tested in the present investigation.

The mechanism of repair of UV lesions has recently been extensively studied by Setlow and Carrier (19) and by Boyce and Howard-Flanders (10); profound differences in the ability to repair UV damage were observed among different strains of *E. coli*.

In this study, the ability of 5-fluorouracil to induce resistance to UV light was compared in various *E. coli* strains having different sensitivity to UV light. The results obtained show that pretreatment of *E. coli* with 5-fluorouracil does not influence the dark-repair mechanism; however, it does affect postirradiation cell division.

MATERIALS AND METHODS

Organisms. The bacterial strains used in this investigation were *E. coli* B and *E. coli* B/r (kindly supplied by E. Witkin); *E. coli* B_{s-1} (obtained from R. Hill); *E. coli* K-12 AB1157, a *uvr*$^+$ strain; *E. coli* K-12 AB1886, a *uvr*$^-$ strain; *E. coli* K-12 AB1899, a *lon*$^-$ strain; and *E. coli* K-12 AB2463, a recombination-deficient strain. All of the K-12 strains were kindly supplied by P. Howard-Flanders. *E. coli* K-12 MM152, another recombination-deficient strain, was obtained from M. Messelson.

Media. The liquid medium for the *E. coli* K-12 strains was minimal medium supplemented with 2.5 mg of Casamino Acids per ml and 10^{-5}% thiamine (13). *E. coli* B strains were grown on M9 medium.

Growth conditions and irradiation. The bacteria were grown and irradiated as previously described (9). Before and after irradiation, viable count was determined on complete medium containing yeast extract and tryptone (YET-agar), or on selective minimal medium-agar (14).

Microscopy. Growth and division of irradiated cells were observed on agar-covered microscope slides as described by Jagger et al. (16) and Adler and Hardigree (3).

5-Fluorouracil was a gift of the Hoffman-La Roche Co., Basel, Switzerland.

RESULTS

The ability of 5-fluorouracil to induce resistance to UV irradiation in *E. coli* strains that differ in their sensitivity to UV light was determined (Table 1). These experiments were performed to determine whether preincubation with the ana-

logue affects the dark-repair mechanism. The characteristic properties of the *E. coli* strains employed are shown in Table 1. These strains were exposed in logarithmic phase of growth to 1 μg of 5-fluorouracil per ml for 45 min, and irradiated; survival was then determined.

5-Flourouracil did not induce resistance to UV light in all of the UV^R strains tested. Only UV^R strains that form filaments after irradiation respond to pretreatment with the analogue. These results show that, in strains of *E. coli* not forming filaments, the dark-repair mechanism is not affected by preincubation with 5-fluorouracil. It thus appears that the analogue may act upon a process specificially concerned with postirradiation filament formation rather than on the dark-repair process.

TABLE 1. *Effect of preincubation with 5-fluorouracil (FU) on survival of different strains of Escherichia coli after irradiation*

Strain	UV[a]	REC[b]	FIL[c]	Survival (%)[d]	
				−FU	+FU
B	UV^R	+	+	8.7	46.0
				12.0	50.0
B/r	UV^R	+	−	32.0	32.0
				38.0	40.0
B_{s-1}	UV^S	+	−	0.075	0.08
				3.6	3.1
K-12 AB1157[e]	UV^R	+	−	51.0	77.0
				50.0	72.0
K-12 AB1886	UV^S	+	−	15.0	25.0
				15.0	20.0
K-12 AB1899	UV^R	+	+	16.0	60.0
				6.0	30.0
K-12 AB2463	UV^R	−	−	7.2	8.0
K-12 MM152	UV^R	−	−	1.7	1.7
				0.3	0.2
W	UV^R	+	+[f]	30.0	73.0

[a] UV^R denotes ability to repair UV-irradiation damage; UV^S denotes mutant strains that are unable to repair UV lesion.

[b] Minus denotes defect in ability to form genetic recombinants (12). These strains are sensitive to irradiation; however, repair of photochemical damage seems to occur (11).

[c] Plus denotes filament formation after UV irradiation.

[d] Values taken from two separate experiments. Since considerable differences in sensitivity to UV light exist in the various strains, the irradiation doses employed varied for the different strains. In strains B_{s-1}, K-12 AB1899, and MM152, survival at two different irradiation doses is shown. In each experiment, however, the cultures grown in the presence or absence of 5-fluorouracil were irradiated with the same dose.

[e] All of the K-12 strains require threonine, leucine, proline, histidine, arginine, and thiamine for growth. Occasionally, FU reduced drastically the viable count of unirradiated K-12 strains on YET-agar. This occasional reduction in survival seems to be due to an effect of FU on cell wall synthesis (21) and was never observed in *E. coli* B.

[f] *E. coli* W forms filaments after UV irradiation; however, the filaments are shorter than those formed in *E. coli* B or *E. coli* K-12 AB1899.

It is of interest to point out that a similarity exists in the UV survival curves of growing and resting filament-forming *E. coli* strains, and growing and resting recombinant-deficient mutants (17). In view of this similarity, the effect of preincubation with 5-fluorouracil on these mutants was also tested; no effect on survival was observed (Table 1).

It has recently been suggested that, in *E. coli* strains that form filaments after irradiation, the cell division mechanism is particularly radiation-sensitive (4). It was therefore of interest to determine whether 5-fluorouracil acts upon this process. A direct proof that preincubation with the analogue indeed affects the postirradiation cell division mechanism of the *E. coli* K-12 *lon* strain and of *E. coli* B could be obtained by microscopy. In these experiments, filament-forming strains grown in the presence or in the absence of 5-fluorouracil were irradiated, and the subsequent growth and cell division of these strains on complete solid medium was compared. Preincubation with the analogue considerably reduced filament formation after UV irradiation of these strains (Fig. 1 and 2).

It has been shown that a variety of postirradiation treatments affect the viability of filament-forming strains. It was of interest to determine whether the action of 5-fluorouracil and postirradiation treatment overlap. In *E. coli* B, the effects of preincubation with the analogue and postirradiation treatment were found to be additive (Table 2). In contradistinction to *E. coli* B, the effects of the two treatments were found to overlap to a large extent in *E. coli* K-12. The reason for this difference in the behavior of those two strains is not yet understood.

DISCUSSION

The experiments reported in the present study show that preincubation with 5-fluorouracil induces resistance to UV light only in certain strains of *E. coli*. The strains affected exhibited high sensitivity to UV irradiation because of postirradiation filament formation. Howard-Flanders et al. (13, 14) have shown that, in *E. coli* K-12, filament formation and increased radiation sensitivity are controlled by the same genetic locus (*lon*). The *lon*$^-$ strains of *E. coli* K-12 are similar in radiation sensitivity and filament forma-

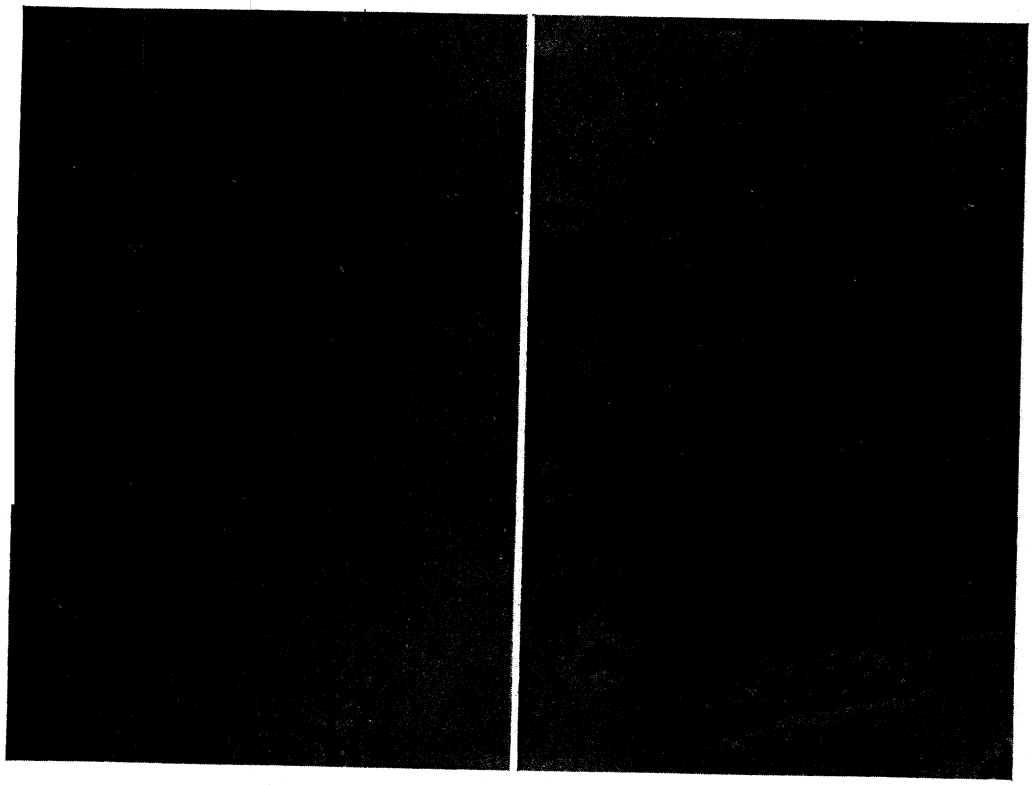

FIG. 1. *Filament formation by irradiated Escherichia coli K-12 AB1899. (left) After irradiation with a dose of 400 ergs/mm², the organism was grown for 4 hr on YET-agar. (right) Preincubated with 1 μg of 5-fluorouracil per ml for 45 min, irradiated with 400 ergs/mm², and grown for 4 hr on YET-agar.*

TABLE 2. *Effect of preincubation with 5-fluorouracil (FU) and postirradiation treatment on survival of Escherichia coli B and E. coli K-12 AB1899 after irradiation*[a]

Strain	Survival (%) on YET-agar		Survival (%) on minimal agar		Survival (%) on YET-agar (first 6 hr at 42 C)	
	−FU	+FU	−FU	+FU	−FU	+FU
B	15	58			64	98
	8	46	32	82	54	81
	4	22	22	60		
K-12 AB1899	12	50	50	73		
	6	30	44	44	40	43
	18	54	51	50		

[a] The strains were grown on minimal medium and, in logarithmic phase of growth, pretreated for 45 min with or without FU (1 μg/ml), and irradiated. Survival was determined after 24 hr of incubation at 37 C. The same UV irradiation doses were used in each separate experiment. (Each line denotes a separate experiment.)

tion to *E. col* B *fil*$^+$ (18). It has been suggested by Adler and Hardigree (3) that in these strains the mechanism of cell division and in particular cross plate formation (cytokinesis) is particularly radiation-sensitive. The mechanism responsible for this irradiation damage is at present unknown. The experiments of Howard-Flanders et al. (14) with irradiated T1 phage suggest that, in the *lon* mutants of *E. coli* K-12 (*uvr*$^+$), the ability to repair photochemical damage in the phage DNA is not impaired. Setlow and Carrier (19) observed that, in *E. coli* B, thymine dimer excision occurs at a rate comparable to that of *E. coli* B/r. Furthermore, the UV-induced inhibition of DNA synthesis in *E. coli* B is resumed after an initial lag, indicating that the excised regions are repaired (20). In view of these results, it seems that the dark-repair mechanism responsible for resumption of DNA synthesis is not impaired in the filament-forming strains.

The radiation sensitivity of the *lon*$^-$ and *fil*$^+$ strains is markedly affected by postirradiation growth conditions. Thus, pantoyl lactone (2, 22), incubation at high temperature (7), liquid holding of the irradiated bacteria before plating (16), and

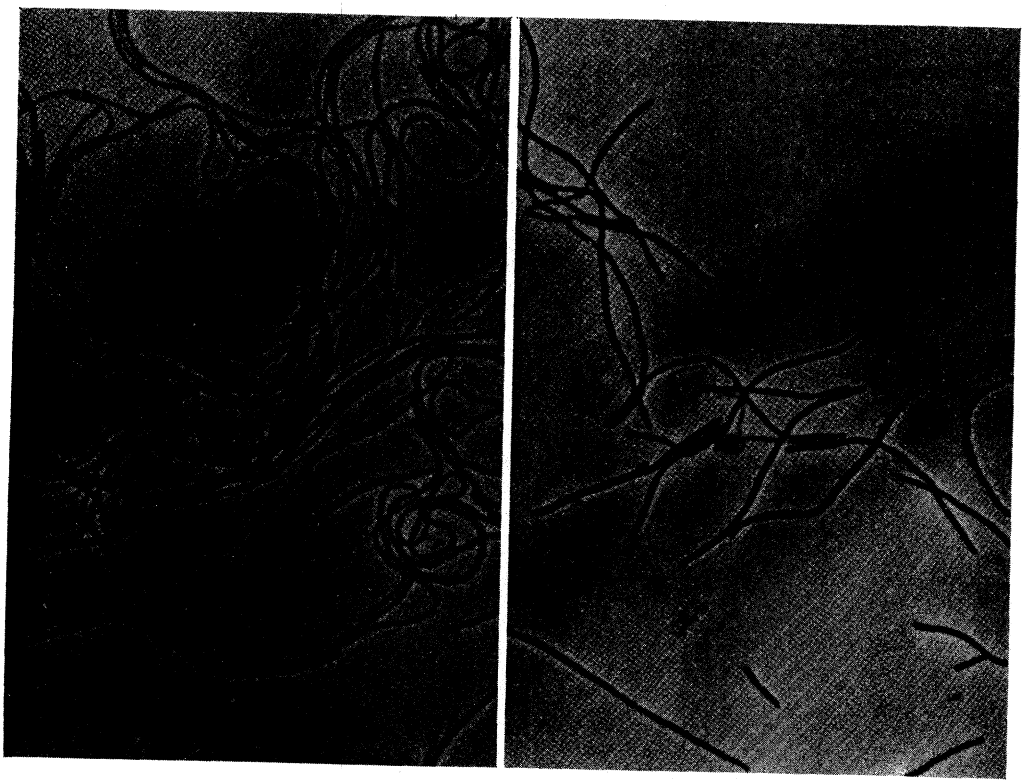

FIG. 2. *Filament formation by irradiated Escherichia coli B. (left) After irradiation with a dose of 200 ergs/mm², the organism was grown for 4 hr on YET-agar. (right) Preincubated with 1 μg of 5-fluorouracil per ml for 45 min, irradiated with 200 ergs/mm², and grown for 4 hr on YET-agar.*

postirradiation growth on minimal medium (5) increase the survival of irradiated bacteria. The increased survival observed after liquid holding, or growth on minimal medium, seems to be due to a delay in postirradiation growth (6, 15).

In the experiments described in Table 2, analogue-grown bacteria were subjected after irradiation to growth conditions which are known to increase the survival of irradiated cultures. These experiments were performed to determine whether pretreatment with 5-fluorouracil may affect irradiation damage in the same way as different postirradiation conditions. The results obtained are, however, inconclusive since 5-fluorouracil-pretreated *E. coli* K-12 *lon* cultures differ from pretreated *E. coli* B cultures in their response to additional postirradiation treatment.

It has previously been shown that preincubation of *E. coli* with 5-fluorouracil inhibits postirradiation protein, RNA, and DNA synthesis (9). This inhibition causes a delay in cell growth and may therefore be responsible for the higher survival observed after UV irradiation.

A similar delay in postirradiation growth may also be responsible for the increased survival observed in *E. coli* B subjected to a nutritional shift from rich to minimal medium, or pretreated with chloramphenicol, prior to irradiation (8).

Adler et al. (1) have recently shown that a partially purified extract of *E. coli* induces division and colony formation in radiation-damaged *E. coli* K-12 *lon* strains. It seems, therefore, that the formation of an unidentified factor may be the rate-limiting reaction in cytokinesis of the radiation-damaged filament-forming strains. Conditions that cause delay in postirradiation growth, such as preincubation with 5-fluorouracil, may allow the synthesis of this factor to proceed to levels sufficient to induce cross cell wall synthesis before cell death occurs.

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